ORIGINAL ARTICLE

Effect of Vitamin C on Monosodium Glutamate (Ajinomoto) Induced Changes in the Ovary of Rats

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ABSTRACT

Objective: To determine the protective effect of Vitamin C on MSG (Ajinomoto) induced histomorphological changes in rat ovary.

Study Design: It was randomized control trial.

Place and Duration of Study: This study was carried out in the department of Anatomy, Islamic International Medical College, Rawalpindi, in collaboration with National Institute of Health (NIH), Islamabad. The study was conducted from September 2015 to March 2016.

Materials and Methods: Total 45 female rats (Sprague Dawley) were used and divided into 3 groups. The control group (C) was kept on plain water and normal laboratory diet, while the experimental group A, was given MSG and experimental group B, was fed on MSG and Vitamin C both, for 4 weeks to induce histological changes in ovary. All rats were sacrificed after 4 weeks. Ovaries were dissected out and examined for gross and histological parameters. Results were compared with the control and experimental groups.

Results: In experimental group A, weight of the ovary was increased as compared to the control group. The histological examination of ovary showed granulosa cell degeneration and decrease in the number of primary follicles in group A. In addition to MSG, vitamin C was also given to experimental group B by mixing it in drinking water and the dissected ovary of this group showed changes which were less severe than experimental group A.

Conclusion: Protective effect of vitamin C is proved on MSG induced histomorphological changes in ovary of rats.

Key Words: *Degenerated Granulosa Cells, Infertility, MSG, Number of Primary Follicles, Vitamin C.*

Introduction

Fertility or fecundity is defined as the ability to conceive children[1],while infertility is defined as inability of a couple to conceive after a period of twelve months of unprotected sexual intercourse. According to WHO, infertility is defined as inability of a couple to conceive after twenty four months of unprotected intercourse.[1] Rate of infertility has increased remarkably over the past twenty years.1Almost one in six couples have a delay in conception while only few of them would attempt for medical treatment.[1]

It is roughly calculated that in 40% to 50% of couples, infertility is due to female problems which include disorders of oocyte production, fallopian tube function and implantation of embryo.[2] According to WHO, the factors responsible for infertility in females include tubal factors 36% ,disorders of ovulation 33% and endometriosis 6% and evincible causes 40%.[3]

Globally the rate of infertility is about 10–15%.[1] Inspite of the fact that Pakistan is one of the popular countries of the world and its population growth rate is about 2% and it also has high rate of infertility that is about 21.9% out of which 3.5% are due to primary causes and 18.4% are due to secondary reasons. According to the statistics ratio this indicates that the average number of children in married Pakistani women is 6.5.[1]

The female reproductive system is easily offended by harmful environmental factors which include environmental chemicals, industrial pollutants and food additives.[1-3]One of the food additive is Monosodium glutamate, commonly known as Ajinomoto.[1-3]

It is commonly used in restaurants (especially mixed in noodles), packaged food industries including chips, salads, soups, canned food and household kitchen.[1-3] MSG increases the palatability of meal by

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Funding Source: NIL ; Conflict of Interest: NIL
Received: Mar 25, 2016; Revised: Apr 07, 2016
Accepted: May 09, 2016

triggering the taste buds to ''UMAMI'' taste which is a pleasant savory taste4and it also accelerates appetite centre which results in increase in body weight.[5]

MSG is a sodium salt of glutamic acid which is one of the naturally occurring non essential amino acid.[6] The part of MSG that negatively affects the human body is glutamate not the sodium.[7,8] It is composed of 78% glutamic acid, 22% sodium and water.[9]

Vitamin C defends the human body against oxidative damage by preventing oxidative damage to DNA, proteins and lipids.[8] Reactive oxygen species (ROS) plays a critical role in the normal function of reproductive system and also in the pathogenesis of female infertility.[10] Oxidative stress will impair the oocyte maturation and folliculogenesis.[11] Treatments that reduce the oxidative stress may help to reduce the infertility caused by this imbalance.[10] This research was done with an objective to observe the reverse histological effects of vitamin C on MSG induced changes in ovary.

Materials and Methods

It was a randomized control trial. The study was carried out in the department of Anatomy Islamic International Medical College in collaboration with National Institute of Health and the duration of study was from September 2015 to March 2016. Forty five female adult rats (Sprague Dawley) with a weight of 250-350g and age between 10-12 weeks were included in this study. Pregnant female rats and rats with any obvious pathology were excluded. Male rats were also excluded. Rats were purchased from animal house of NIH, Islamabad where they were kept under standard laboratory conditions.

Rats were randomly divided into 3 groups. The control group C (n = 15) was kept on standard pellet diet with tap water to drink. The experimental group A (n = 15) was fed on MSG diet for 4 weeks to induce degenerative changes. The experimental group B (n = 15) was fed on MSG and vitamin C diet for a period of 4 weeks.

Dissected ovaries were examined for gross and histological parameters.

After tissue processing, embedding was done in paraffin wax. For the preparation of slides haematoxylin and eosin stains were used. Microscopic study was done under 10x and 40x objective. Statistical analysis was done in SPSS version 20.0. Qualitative variables were analyzed in percentages and compared by applying Pearson Chi Square test. Quantitative data was expressed as Mean + S.D. The means were compared among three groups by using one way Analysis of Variance (ANOVA). A p–value of <0.05 was considered statistically significant.

Results

Gross Assessment

Weight of Ovary: Weight of ovaries and histological analysis of all experimental animals were done. The weight of ovary in control group was 0.15±0.02 gm. The weight of ovary in group A, was 0.21±0.03gm. The weight of ovary in group B, was 0.18 ±0.05gm.So the mean weight of ovary was greater in group A and the difference was statistically significant ($p < 0.001$). (Table I)(Fig 1).

Control group had significantly lower mean weight as compared to experimental group A ($p< 0.001$). Control group had lower mean weight as compared to experimental group B ($p = 0.03$) which is insignificant. Similarly experimental group A had higher weight as compared to experimental group B ($p = 0.06$) which is also insignificant.

Table I: Mean weight of ovaries in three groups

Groups	Weight of ovary Mean±SD	SEM	p- value
Control Group C (n = 15)	0.15 ± 0.02	0.005	< 0.001
Group A (n = 15)	0.21 ± 0.03	0.009	
Group B (n = 15)	0.18±0.05	0.01	

Microscopic Assessment

One quantitative and two qualitative histological parameters were also studied.

Number of primary follicles

Number of primary follicles were counted per slide at 10x.The average number of primary follicles were 5.9±1.2 in control group C, In experimental group A, the number of primary follicles is 2.7±1.8 and in experimental group B, the number of primary follicles is3.4±1.7 and this difference of mean is statistically significant (p-value<0.001). (Table II and Fig 1).

Control group had significantly higher mean of

number of primary follicles as compared to experimental group A (p< 0.001). Control group had higher mean of number of primary follicles as compared to experimental group B (p = 0.001) which is insignificant. Similarly experimental group A had lower mean of number of primary follicles as compared to experimental group B (p = 0.28) which is insignificant.

Table II: Mean number of primary follicles in three groups

Groups	Number of primary follicles Mean ± S.D	SEM	p-value
Group C (n = 15)	5.9 ± 1.2	0.30	< 0.001*
Group A (n = 15)	2.7 ± 1.8	0.46	
Group B (n = 15)	3.4±1.7	0.44	

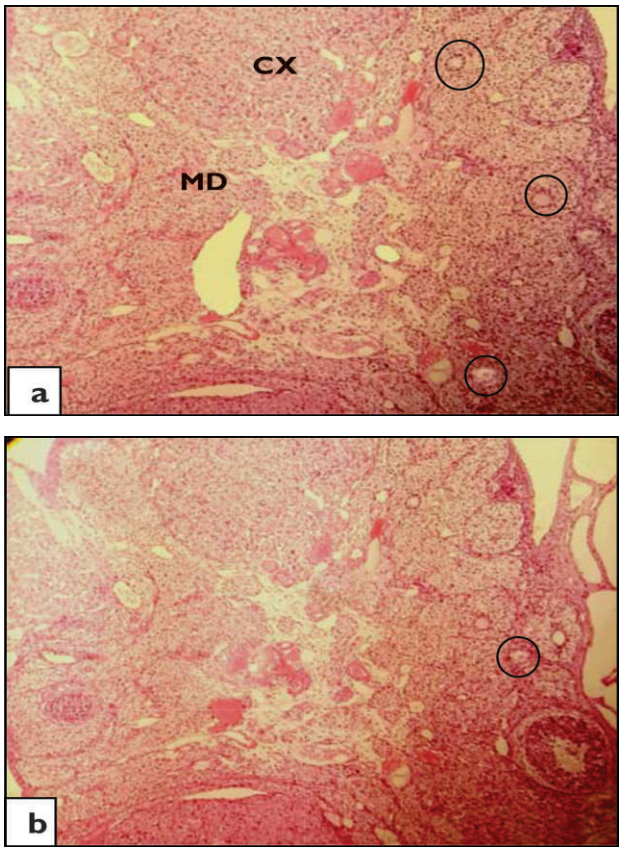

Fig 1: Photomicrograph (a) showing cortex (CX), medulla (MD) and primary follicles in animal rat no. C13 and (b) showing primary follicle in animal rat no. A1 (demarcated by a circle). H & E Stain, x10

Granulosa cell degeneration

In control group C, none of the rat showed granulosa cell degeneration. In group A, 11 rats (73.3%) showed granulosa cell degeneration and in group B, there were 6 rats (40%) which showed degeneration of granulosa cells. Presence of granulosa cell degeneration was significantly (p-value <0.001) different between all groups.(Fig 2).

Vascular congestion in medulla

In control group C, none of the rat was found with increase vascular congestion in medulla whereas in group A, there were 12 rats (80.0%) with an increase in vascular congestion and in group B, there were 6 rats (40%) with increased vascular congestion. Statistically significant p-value <0.001 was obtained after comparison between all groups.(Fig 3).

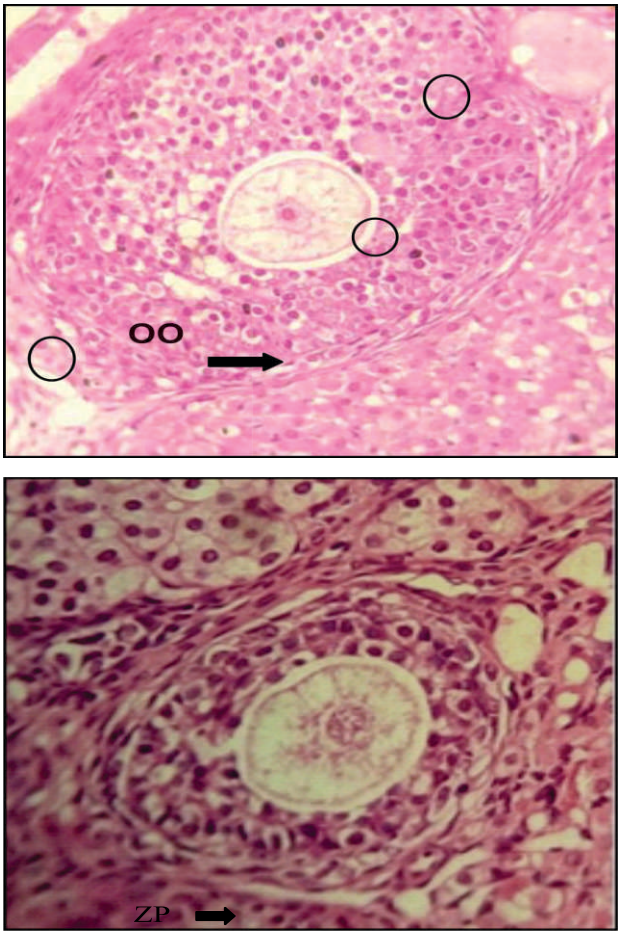

Fig 2: Photomicrograph (a) showing oocyte(OO), zona pellucida (ZP)and granulosa cell degeneration in animal rat no. A3(demarcated by a circle) and (b) absent degeneration in animal rat no. B4. H & E Stain, x40.

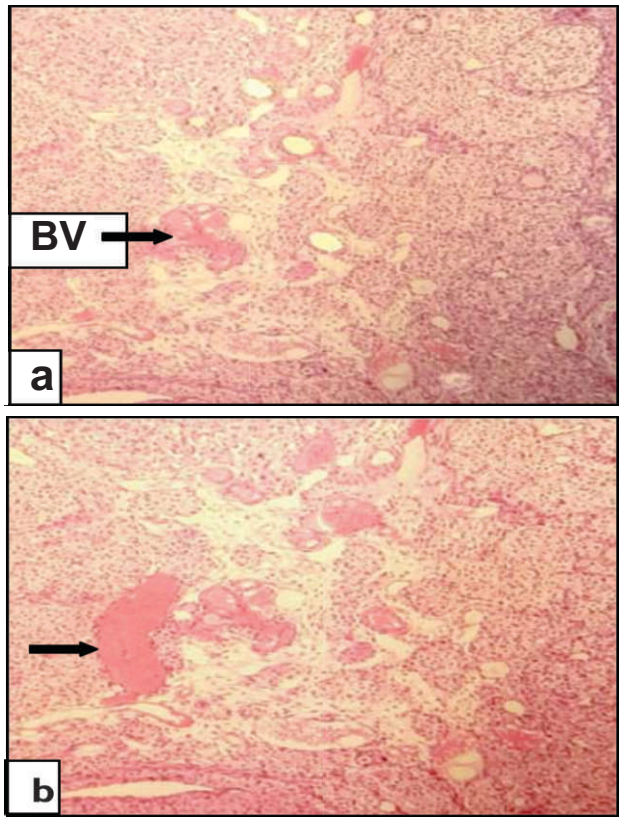

Fig 3: Photomicrograph (a) showing congested blood vessel (BV) in animal rat no. C5 and (b) showing vascular congestion in animal rat no. A2.H & E Stain, x10.

Discussion

The effect of MSG on the female reproductive system causes degenerative histological changes in ovaries causing granulosa cell degeneration, vacuolization of theca cells and decrease in the number of primary follicles.[10-12]

In the conducted study, it was observed that there was significant increase in the weight of ovaries in group A in which the average weight was 0.21 g which was significantly different from the control group (0.15g). The result of this study disagree with the previous report of Ilegbedion IG et al[13] and Das[14], showing insignificant increase in ovary weight due to MSG intake for a period of 2 and 10 weeks respectively, although it was also dose dependant In group B the average weight was 0.18 g, lower than that of group A. Decrease in the weight of ovary in group B may be attributed to the effective anti oxidating properties of vitamin C.[2]

Primary follicle is defined as an oocyte surrounded by a single or double layer of cuboidal epithelium.[8] The number of primary follicles were counted in this study, the average number of primary follicles in group A is 2.7 and in group B is 3.4. In comparison, the number of primary follicle in group C is 5.9. The result of this study disagree the previous report of Das[14], in which the number of primary follicles were increased in the experimental group. Naureen et al reported similar results in the study of lead induced changes in ovary of mice.[7,8]

Folliculogenesis was improved in group B, because vitamin C improves the integrity of follicular membrane and also responsible for the synthesis of collagen, required for the growth of follicles.[2]

In the present study, 73.3 % of the rats in group A and 40% of the rats in group B also showed granulosa cell degeneration which was highly significant from the control group in which none of the ovary showed such pathological change. Result of this study is in agreement with similar studies by Veronika Husarova[9], Eweka et al [10,11], Saber A. Sakr[12] , Ahmed et al[15] in which MSG was given for a period of 2 weeks. Similar study was carried out by Afeefy et al in which MSG was given for a period of 4 weeks, causes degenerative and atrophic changes in kidneys.[16]

Degeneration of the granulosa cells can be due to the toxic effects of MSG.[17] Reduction in the degeneration of granulosa cells in group B can be attributed to the wound repair ability of vitamin C by stimulating the synthesis.[18] Vascular congestion is defined as blood filled dilated veins.[19] 80% of the rats in group A and 40% in group B, showed increase vascular congestion which was highly significant from the control group in which none of the rat showed increase vascular congestion. The results of this study is consistent with the previous work of Mustafa et al.[20] Similar work has been done in Nigeria by Oladipo et al in 2015, in which vascular congestion was observed in experimental group with the use of MSG for 2 weeks.[21]

Vascular congestion was ameliorated in experimental group B, because vitamin C protects the capillary endothelium from oxidative damage by increasing the synthesis of nitric oxide.[22,23] collagen and extracellular matrix.[24,25]

The results of this study proved the alternate hypothesis that states that vitamin C has protective effect on MSG induced histological changes there by rejecting the null hypothesis.

Limitation of Study: Estrous cycle was not traced in

the current study.

Conclusion

Protective effect of vitamin C is proved on MSG induced histomorphological changes in ovary of rats.

Recommendations

Effect of MSG can also observe on other organs. Duration and sample size of study can also be increase.

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